Thermal cyclers: key thermal cycling concepts and ramp rates

Key thermal cycling specifications are often found in instrument literature and on the Web with very little explanation of how the numbers are derived or used. Ramp rates, thermal overshoot, and other thermal cycling characteristics are key to PCR performance, and are explained in depth here using a variety of Applied Biosystems™ thermal cyclers as benchmarks. In addition, ramp rates are tested and compared to published specifications.

Ramp rates: maximum versus average, and block versus sample

Temperature cycling is fundamental to all PCR reactions, and how fast an instrument can ramp between temperatures will dictate the overall speed of the PCR run. Because of this, thermal cycler manufacturers publish their ramp rates to indicate the change in temperature over time. Ramp rates are typically expressed in °C/second. Looking at this concept graphically in a plot of temperature versus time, the ramp rate is simply expressed by the slope of the curve. A steeper curve represents a higher ramp rate, and means that a specific temperature range can be covered in a shorter time. The terms "up ramp" and "down ramp" refer to the ramp rate when heating and cooling, respectively. Of course, a faster ramping block will result in a faster PCR run, so many thermal cycler manufacturers seek to show the highest possible ramp rate. However, the way ramp rates are measured may make them appear to be greater than they really are.

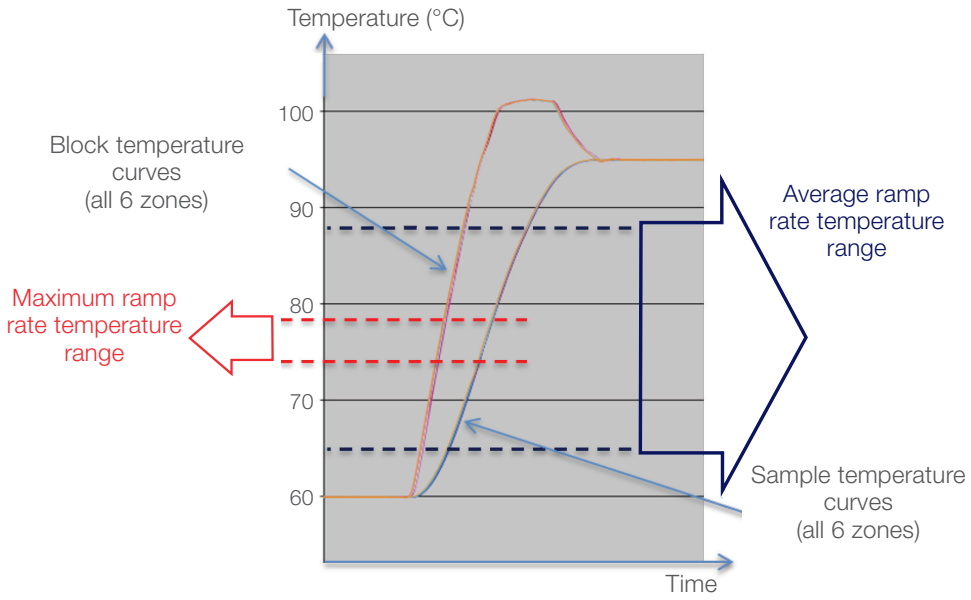

Figure 1. Real block and sample temperature curves from all six zones during up ramp on the Applied Biosystems™ ProFlex™ 96-well PCR system. The dotted lines indicate the temperature range sampled for the maximum ramp rate and the average ramp rate.

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The metal blocks of thermal cyclers must be heated and cooled between steps in PCR, such as denaturation, annealing, and extension. A commonly published specification is the **maximum block ramp rate**, also known as the peak block ramp rate. The maximum block ramp rate corresponds to the highest achievable block performance. This maximum performance may only be achieved during a very brief period during the ramp. Thermal cycler manufacturers may also publish the **average block ramp rate**, which represents the rate of temperature change across a longer portion of the ramp, providing a more representative measurement of the instrument's performance and speed (Figure 1).

However, just because the block is ramping quickly does not mean that the liquid of your PCR reaction (sample) is ramping just as quickly. It takes time for the heat from the thermal cycler's block to be transferred to the sample. Because of this, ramp rates are for Applied Biosystem™ instruments expressed in terms of **average sample ramp rate** or **maximum sample ramp rate**. Ramp rates that are based on the block temperature alone, without accounting for sample volume, do not reflect the ramp rate experienced by the sample.

Applied Biosystems instruments use a proprietary algorithm that calculates the temperature of the PCR sample itself, based on the volume entered. This allows us to publish sample ramp rates. We also publish block ramp rates to allow a more direct comparison to other manufacturers' instruments.

Overview: sample temperature control

How thermal cyclers achieve and measure temperature differs, depending on the manufacturer. Often, thermal cyclers are programmed to achieve temperatures based on block temperature alone, without accounting for the temperature of the liquid sample.

However, just because the block has achieved its desired temperature, or set point, does not mean that the sample has achieved the same set point. The proprietary temperature control algorithm used by Applied Biosystems instruments helps ensure that temperature set points are reached quickly and accurately, by accounting for the sample volume used. This algorithm actually drives the block temperature higher than the set point to permit the sample to reach the set point faster. This is known as the "block overshoot thermal energy", depicted in green in Figure 2. It is important to note that although the block overshoots the set point, the sample should arrive at the set point with no overshoot. In addition, the instrument does not begin to count down the time of that step until the sample reaches the set point. This helps ensure that the sample spends exactly the amount of time at a specific temperature that you programmed into the instrument.

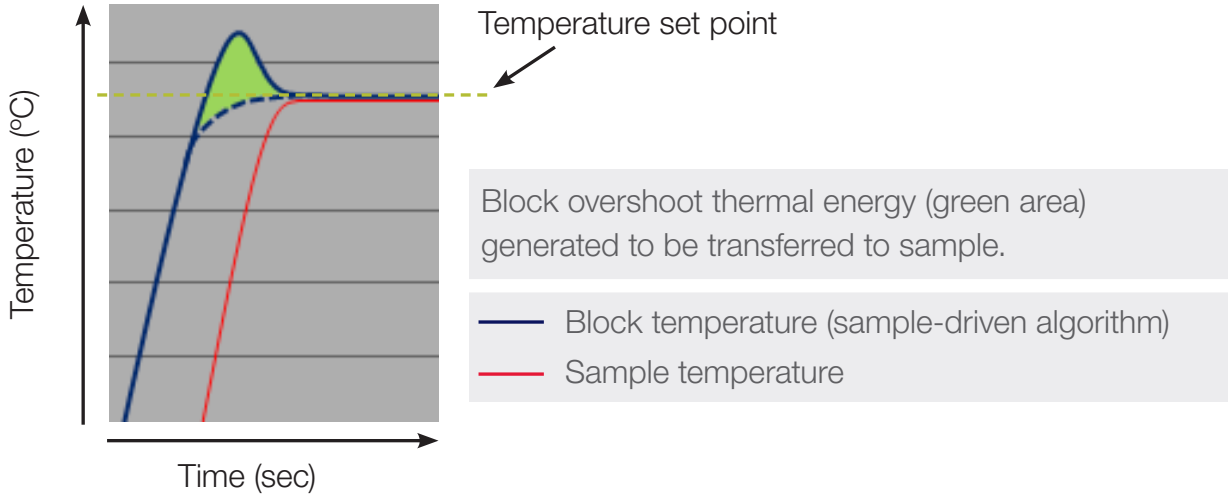

Figure 2. Block overshoot thermal energy. The reaction block (blue) and sample (red) temperature curves and associated block overshoot thermal energy (green) during a heating phase that is ideally controlled. The horizontal dotted line indicates the set temperature for this phase.

Our proprietary temperature control algorithm helps ensure samples reach a stable set point temperature—within a range of ±0.25°C—without overshoot and associated deleterious effects. The algorithm uniquely predicts the temperature and speed of the PCR reaction while accounting for the volume of the PCR reaction and the thickness of the reaction tube. Sample temperature or sample ramp rates are typically not mentioned by other instrument manufacturers.

Published versus measured ramp rates

Our Applied Biosystems™ ProFlex™, Veriti™, and SimpliAmp™ thermal cyclers include both the maximum block ramp rate and the more relevant maximum sample ramp rate. The published data [1–3] are based on the averages of measured performance data captured during product testing (Table 1).

Some manufacturers' published thermal cycler ramp rates are known to vary significantly from actual measured ramp rates [4]. In a recent comparison of maximum block ramp rates of multiple thermal cyclers, the smallest differences between the published and measured ramp rates were observed for Applied Biosystems thermal cyclers (Table 2). These differences are useful when directly comparing instrument capabilities.

Table 1. Measured and published data for ProFlex, Veriti, and SimpliAmp thermal cyclers.

Measured data				
Block type	Max block rate (°C/sec)		Max sample rate at 1 µL (°C/sec)*	
	Up ramp	Down ramp	Up ramp	Down ramp
ProFlex 96-well	6.4	5.9	4.6	4.3
ProFlex 3 x 32-well	6.2	5.6	4.4	4.2
SimpliAmp 96-well	4.0	3.7	3.1	3.0
Veriti 96-well Fast	4.9	5.1	4.3	3.6
Veriti 96-well	3.8	3.9	3.4	2.8
Published data (average of above)				
ProFlex 96-well	6.0		4.4	
ProFlex 3 x 32-well	6.0		4.4	
SimpliAmp 96-well	4.0		3.0	
Veriti 96-well Fast	5.0		4.2	
Veriti 96-well	3.9		3.4	

*Per industry standard practice, a reaction volume of 1 µL is used to show the highest achievable sample ramp rate.

Table 2. Comparison of average maximum block ramp rates (°C/sec) of thermal cyclers from different manufacturers [4].

ProFlex 96-well Thermal Cycler				ProFlex 3 x 32-well Thermal Cycler				Mastercycler™ Pro S Thermal Cycler				C1000 Touch™ Thermal Cycler			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
6.0	6.0	6.4	5.9	6.0	6.0	6.2	5.6	6.0	4.5	5.4	3.9	5.0	5.0	3.0	2.4

SimpliAmp 96-well Thermal Cycler				T100™ Thermal Cycler				MyCycler™ Thermal Cycler				XP Cycler			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
4.0	4.0	4.0	3.7	4.0	4.0	2.6	1.6	2.5	1.5	2.6	1.6	5.0	3.0	2.6	1.8

Touch Thermal Cycler				Mastercycler Nexus Thermal Cycler				Labcycler				Bioer GeneMax			
Published		Measured		Published		Measured		Published		Measured		Published		Measured	
Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
3.0	2.5	3.6	2.9	3.0	2.0	3.3	1.9	4.2	3.6	3.7	3.2	5.0	4.0	4.6	3.5

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Summary

Precise temperature control by a thermal cycler is crucial for the accuracy and efficiency of PCR experiments. Multiple instrument-specific factors, including the actual instrument ramp rates, sample temperatures, and how the thermal cycler ensures accurate overshoot calculation, contribute to the integrity and performance of PCR assays.

Our proprietary algorithm uniquely enables our thermal cyclers to achieve the correct temperatures of the samples, rather than the blocks; the resulting published ramp rates help ensure adherence to the programmed protocol and evaluation of an instrument's true performance.

References

1. ProFlex PCR System User Guide. P/N MAN0007697, Rev 2.0.
2. Veriti Thermal Cycler User Guide. P/N 4375799, Rev. E
3. SimpliAmp Thermal Cycler User Guide. P/N MAN0009889, Rev. A
4. Kim YH, Yang I, Bae YS, Park SR (2008) Performance evaluation of thermal cyclers for PCR in a rapid cycling condition. *Biotechniques* 44(4):495–505.

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